

# Chronological Age-Grading of Three Species of Stored-Product Beetles by Near-Infrared Spectroscopy



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## INTRODUCTION

- The rice weevil, *Sitophilus oryzae* (L.), the lesser grain borer, *Rhyzopertha dominica* (F.), and the red flour beetle, *Tribolium castaneum* (Herbst), are three of the most important pests of stored grain and processed grain products in the U.S.
- Successful management of these pests requires thorough sampling protocols and subsequent decision-making based on predictive population models or expert system analysis.
- Because oviposition by these species is not temporally uniform, the accuracy of predictive models used to manage these species can be improved significantly if the age-structure of the pest population is incorporated.
- However, except for *S. oryzae*, there is no information on methods to determine the chronological or physiological age of these Coleopterans.
- We previously showed that near-infrared spectroscopy (NIRS), a rapid procedure, can be used to determine chronological age of the house fly, a relatively short-lived Dipteran.
- The objectives of this study were to determine if NIRS could be used for determination of chronological age in these three long-lived species of beetles, to determine the role of cuticular lipids in the ability of NIRS to age-grade adult *S. oryzae*, and to determine whether water content in adult weevils varies with age and if NIR wavelengths that are absorbed by water have any effect on the ability to determine age.

## METHODS

- A near-infrared spectrometer (DA7000, Perten Instruments) (Fig. 1A) was used to collect spectra from single adults that were placed in a V-shaped trough (Fig. 1B). An 8-mm diameter fiber optic probe illuminated the insect, and a 2-mm diameter optic probe carried reflected energy from the insect to the spectrometer (Fig. 1C).



Fig. 1A. NIR diode array light source



Fig. 1B. Single insect viewing area

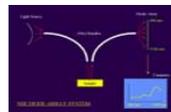


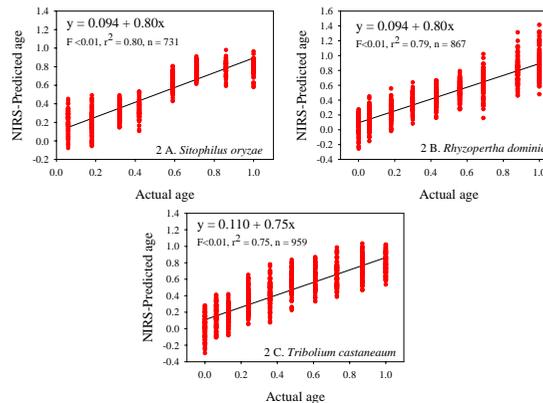
Fig. 1C.

- We used a physiological age scale, rather than days, in the analysis because the three species live for different lengths of time when maintained at the same temperature. Thus, an insect of age 0 is a newly emerged adult, and an insect of age 1 has reached its maximum life span.
- To test the role of cuticular lipids on NIRS age-grading predictions, weevils with and without cuticular lipids were used to develop NIRS calibration models and these models were validated by predicting the age of another set of weevils.
- Water content on weevils of five different age groups was determined by the oven method.

## RESULTS

### NIRS AGE-GRADING

- There was a strong relationship between NIRS-predicted age and the actual chronological age of the insects for all the three species (Figs. 2A, 2B, and 2C).
- Confidence limits of the NIRS-predicted ages ( $\pm 0.3$ ) were similar in the models developed for each of the three species. Classification accuracies in the validation tests ranged from 82 to 92%.



### EFFECTS OF CUTICULAR LIPIDS ON AGE-GRADING

- Although the relationship between NIRS-predicted age and actual age was significant in both unextracted and hexane-extracted weevils, confidence limits were smaller and  $r^2$  value was higher for the calibration model developed with unextracted weevils.

- Classification accuracies in the validation sets were 79 and 44% from the unextracted and hexane-extracted models, respectively.
- The difference spectrum (Fig. 3) shows higher absorbance at all wavelengths above 900 nm for the unextracted weevils. These wavelengths correspond to lipids.

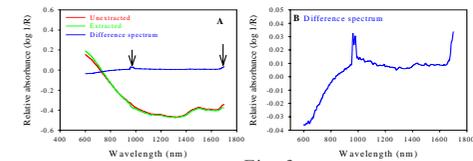
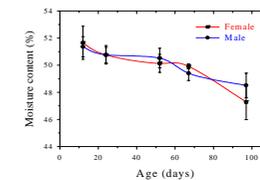


Fig. 3

### EFFECT OF WATER CONTENT

- Water content varied with age but not with sex. Younger weevils tended to have higher water content than older weevils in both sexes (Fig. 4).
- Excluding wavelengths associated with water from the NIRS models slightly reduced the  $r^2$  value, increased the value of confidence limits, and decreased the accuracy of classification from 83% to a 64%.



## SUMMARY

- When life spans are normalized on a scale from 0 to 1, the confidence limits on predicted ages for unsexed adults of each species were about  $\pm 0.3$ . Thus, younger adults within the first one-third of their life can be easily differentiated from older adults
- Based on beta coefficients, absorbance regions corresponding to  $\text{CH}_3$ ,  $\text{CH}_2$ , and  $\text{CH}$  groups were the most important for NIRS age-grading in the three species. These methyl groups are common constituents of most insect cuticular and internal lipids
- Our results provide evidence that these compounds have a significant role in NIR absorption and NIR age classification
- Excluding wavelengths associated with  $\text{H}_2\text{O}$  absorbance reduced the percentage of correct age classification